A single cell approach to problems of cell lineage and commitment during embryogenesis of *Drosophila melanogaster*

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Summary

The mechanisms leading to the commitment of a cell to a particular fate or to restrictions in its developmental potencies represent a problem of central importance in developmental biology. Both at the genetic and at the molecular level, studies addressing this topic using the fruitfly *Drosophila melanogaster* have advanced substantially, whereas, at the cellular level, experimental techniques have been most successfully applied to organisms composed of relatively large and accessible cells. The combined application of the different approaches to one system should improve our understanding of the process of commitment as a whole. Recently, a method has been devised to study cell lineage in *Drosophila* embryos at the single cell level. This method has been used to analyse the lineages, as well as the state of commitment of single cell progenitors from various ectodermal, mesodermal and endodermal anlagen and of the pole cells. The results obtained from a clonal analysis of wild-type larval structures are discussed in this review.

Key words: *Drosophila melanogaster*, cell lineage, commitment, single cell approach, clonal analysis.

Introduction

Studies on fate mapping and cell lineage are of special interest because they permit us to investigate the mechanisms by which a cell is committed to its fate. A fate map is a diagram that shows what will become of each region of the embryo in the course of normal development. Cell lineage analysis is a form of fate mapping in which a cell is labelled at an early stage followed at a late stage by the identification of the position and the cell types of its progeny (Slack, 1983). During the last two decades efforts in this field have received considerable support by the introduction of methodological improvements and innovations.

Three main approaches have been used. The first is to directly follow the development of cells *in vivo*. In *Caenorhabditis elegans* this approach has allowed the elucidation of the entire cell lineage. This small and transparent worm consists of an invariable number of cells (e.g. Sulston, Schierenberg, White & Thomson, 1983; Sternberg & Horvitz, 1984). The second type of approach uses lineage tracers. Labelling may be achieved by intra-cellular injection of a marker enzyme (HRP) or a fluorescent dye into identified early embryonic cells. This method has been mainly applied to lineage studies on the embryonic development of frogs (Heasman, Wylie, Hauser & Smith, 1984; Jacobson, 1985), leeches (Weisblat, Sawyer & Stent, 1978; Weisblat, Zackson, Blair & Young, 1980) and ascidians (Nishida & Satoh, 1983, 1985). Similarly, labelling small numbers of early gastrula cells by injecting HRP has been used for fate mapping in *Drosophila* (Technau & Campos-Ortega, 1985). The development of genetically labelled cells has been studied in genetic mosaics. The gynandromorph technique, consisting of chromosome loss in the first mitotic division, has been applied to fate mapping in *Drosophila* (for review, see Janning, 1978). A different way of producing mosaics is to induce mitotic recombination during the proliferation period with X-rays (discovered by Stern, 1936). This technique has been used extensively for the delineation of the
boundaries of clonal restrictions in \textit{Drosophila} (e.g. Garcia-Bellido, Ripoll & Morata, 1973; Weischaus & Gehring, 1976; Lawrence & Morata, 1977; Campos-Ortega & Waite, 1978). Finally, mosaics (chimaeras) may be produced microsurgically by mixing cells derived from genetically different individuals (\textit{Drosophila}: Illmensee, 1978; mouse: Gardner, 1968). The third approach consists of ablating cells and analysing the resulting structural deficiencies (Lohs-Schardin, Sander, Cremer, Cremer & Zorn, 1979; Underwood, Turner & Mahowald, 1980) or interactive or regulative processes (Sulston & White, 1980; Shankland & Weisblat, 1984; Doe & Goodman, 1985).

On its developmental pathway, a cell acquires different states of commitment via a stepwise restriction of its developmental potencies. The fate map itself does not provide any information about the state of commitment of cells in a particular region of the embryo, but the existence of a fate map is a prerequisite for the interpretation of nearly all experiments concerned with early developmental decisions. By perturbing the normal course of development, experimental embryology tries to elucidate the mechanisms leading to pattern formation. Commonly, the state of commitment is tested by removing a cell or a piece of tissue from its original position and by following its development either \textit{in vitro} or \textit{after} transplantation into a different environment \textit{in vivo}. This kind of experiments has been applied mainly to embryological studies of vertebrates, especially in amphibians (Mangold, 1933; Holtfreter, 1925; Nakamura & Takasaki, 1970; Heasman \textit{et al.} 1984; Heasman, Snape, Smith & Wylie, 1986) and to the mouse (Tarkowski & Wroblewska, 1967; Kelly, 1977; Gardner, 1968, 1984).

The fruitfly \textit{Drosophila melanogaster}, on the other hand, has been extensively used in genetic research for more than 70 years and in view of the rapid progress in molecular biology one would expect the first clues to the mechanisms of commitment at a genetic level to come from this species. The only disadvantage \textit{Drosophila} seems to have is its small size. For this reason, experimental embryological studies on \textit{Drosophila} are rarely found and most of them are based on multicellular approaches. Since it may well be impossible to elucidate the mechanisms of commitment by exclusively dealing with the genetic aspects of the problems there is a real need for experimental techniques on \textit{Drosophila} at the cellular level. The most direct evidence as yet on determinative decisions in \textit{Drosophila} was provided by transplanting genetically labelled nuclei or cells into hosts of different genotypes (Zalokar, 1971; Illmensee, 1972; Kauffman, 1980; Simcox & Sang, 1983). These experiments yielded no indication of developmental restrictions at the nuclear level. In contrast, as soon as cells were first formed, restrictions in their developmental potential were also observed (for review see Illmensee, 1978). With respect to the various anlagen these investigations did not allow more precise information on the state of commitment or the mechanisms involved. Furthermore, they were restricted to adult development since most of the markers used were adult cuticle markers. Since in the holometabolous insect \textit{Drosophila} the principles of larval and adult development may differ considerably and, since the most important events with respect to pattern formation occur during early embryogenesis, an experimental design was needed that would enable the experimenter to trace the development of any progenitor cell in any position of the \textit{Drosophila} embryo from the moment of cellularization until the final differentiation of the progeny cells.

A simple method that meets these requirements has been recently developed (Technau, 1986). By analogy with a technique devised for studying the commitment of blastomeres in \textit{Xenopus} (Heasman \textit{et al.} 1984), this method consists of transplanting single cells that are labelled with a lineage tracer. Labelling is achieved by allowing the lineage tracer (e.g. HRP) to become incorporated into all cells during cell formation in a \textit{Drosophila} embryo which then serves as a donor for labelled progenitor cells. The development of any of these cells can be followed after transplantation into a host which is unlabelled. Since a precise fate map of the blastoderm and of the early gastrula stage is available (Technau & Campos-Ortega, 1985; Hartenstein, Technau & Campos-Ortega, 1985), the positions from or to which cells are transplanted can be defined unambiguously according to the location in a given larval organ anlage. A very important feature of the transplantation assay is its experimental flexibility. Cells can be brought into homotopic or heterotopic positions with respect to the various anlagen or they may be transplanted at or between different developmental stages. Furthermore, cells may be transplanted into hosts of different genotypes; mutations affecting regional specification or cell type determination will be of special interest with respect to the mechanisms involved.

Meanwhile, a variety of experiments has been conducted using this method (Technau \& Campos-Ortega, 1986\textit{a},\textit{b}, 1987; Beer, Technau \& Campos-Ortega, 1987). The embryonic development of single progenitor cells from several larval anlagen within the three germ layers and of the pole cells has been followed. So far, the experiments have provided a great deal of information about lineage characteristics and developmental potencies of these cells. Thus, it seems reasonable now to survey the main results of these studies. The purpose of this review is to bring together and discuss aspects of lineage and
commitment of the different types of progenitor cells of the wild-type *Drosophila* embryo.

(1) The experimental design

The method consists of two steps. In the first step, a lineage tracer e.g. horseradish peroxidase (HRP) is injected centrally into an egg before the formation of cell membranes. The marker diffuses rapidly through the entire egg and becomes incorporated into all cells during cellularization. After completion of cellularization, 8–12 min after the start of gastrulation, the cytoplasmic connections between the cells are lost and the marker is trapped intracellularly. In the second step, a few cells are removed from the globally labelled donor from a given position according to the fate map (Technau & Campos-Ortega, 1985; Fig. 1). Then a single cell is transplanted into a well-defined position in an unlabelled host embryo. The characteristics of the progeny cells, such as clone size, distribution and histotypes, can be analysed at later stages of development in whole mounts or in sections (Figs 2, 3). If the cells are simultaneously filled with a fluorescent dye, like FITC–dextran, the success of the introduction of the single cell can be monitored under a fluorescence microscope immediately after the transplantation.

Homotopic transplantations should reflect the normal development of a cell within a given anlage. The progeny of homotopically transplanted cells participate in the formation of the larval organs of the host in agreement with the fate map and they exhibit the characteristic features of cells of these organs. Thus, compared to what is known about normal cellular development during *Drosophila* embryogenesis, transplanted cells seem to differentiate quite normally. Heterotopic transplantations, on the other hand, provide information about the state of commitment of the transplanted cell. Most importantly, they test the role of cell interactions, of positional information and of cell lineage in the process of commitment. To estimate the determined state of a heterotopically transplanted cell, the morphological characteristics of its progeny cells (cell shape, size, location, etc.) are used.

(2) Clonal analysis of ectodermal derivatives

(2.1) *The fate map*

Intracellular injections of HRP immediately before cell closure (small groups of cells become labelled via still existing cytoplasmic bridges) allowed a precise localization of most of the larval anlagen at the beginning of gastrulation (Technau & Campos-Ortega, 1985; Fig. 1B). By taking into account the morphogenetic movements that had already taken place during the first minutes of gastrulation it is possible to extrapolate the state of the map back to the blastoderm stage (Hartenstein et al. 1985; Fig. 1A). By analysing the global mitotic pattern during embryogenesis and by counting cells in the various larval primordia by histological means it was possible to determine the number of precursor cells in most of the larval anlagen (Hartenstein & Campos-Ortega, 1985).

The major portion of progenitor cells (approx. 3800) belongs to the ectoderm, about 700 of which are going to invaginate as a continuous sheath at the anterior and posterior pole to form the fore- and hindgut, respectively. The progenitors of the central nervous system (CNS), on the other hand, segregate from the ectoderm as single cells, the so-called neuroblasts (with the exception of the optic lobe anlagen which invaginate as aggregates). Most of the ectodermal cells remain in the periphery to become epidermis and its annexes. The neural progenitor cells originate from an area called the neurogenic ectoderm. The neurogenic ectoderm, which will give rise to the ventral nervous system – the ventral neurogenic region (vNR) – maps between the anlagen of the mesoderm and of the dorsal epidermis within the segmental anlagen C1–A10. The neurogenic ectoderm which gives rise to the head ganglia – the procephalic neurogenic region (pNR) – maps between the anlagen of the truncal ectoderm and the foregut and it nearly reaches the dorsal midline. The progenitors of the peripheral nervous system (PNS), on the other hand, are found in the entire ectoderm, except in the dorsalmost area of the truncal ectoderm.

Segregation of neuroblasts from the pNR seems somewhat irregular and is difficult to follow. The vNR on both sides of the embryo together contain about 1800 cells of which about 25% segregate as neuroblasts in three waves during germ band elongation – accordingly, they are called SI, SII and SIII neuroblasts (Hartenstein & Campos-Ortega, 1984). HRP injections at several different positions within the neurogenic regions show that putative epidermoblasts and putative neuroblasts are intermingled throughout the entire neurogenic ectoderm. The density of putative neuroblasts within the vNR, however, decreases from ventral to more dorsal positions. Thus, a gradient of neurogenic abilities exists within the vNR.

(2.2) *The degree of commitment of ectodermal cells*

We wondered how the separation of the two different lineages (epidermal and neural) from cells which are in the same immediate neighbourhood is achieved. To what extent are these cells already committed for
Fig. 1. Fate map of the Drosophila blastoderm (A) and early gastrula stage (B). (A) shows a planimetric reconstruction, (B) shows a lateral view of the left half of an embryo. The fate map in B was obtained by intracellularly injecting cells with HRP a few minutes after the start of gastrulation (Technau & Campos-Ortega, 1985). This map was traced back onto the blastoderm (map (A), Hartenstein et al. 1985). Hatched areas in (A) will invaginate at gastrulation. Numbers indicate the size of the different anlagen referring to one half of the embryo at the blastoderm stage as obtained by histological means (Hartenstein & Campos-Ortega, 1985). In order to avoid intercellular diffusion of the label, transplantations of progenitor cells were performed after completion of cell closure at the onset of gastrulation. Therefore, the positions from/to which cells were transplanted refer to fate map (B). The position of a cell is defined by its location relative to the longitudinal and dorsoventral egg axis (given in % egg length, EL and % of the ventrodorsal diameter, VD, respectively). Arrows with numbers indicate the modes of transplantation discussed in the text. Stippled areas designate the neurogenic regions. am, anterior midgut; as, amnioserosa; CF, cephalic furrow; cl, clypeolabrum; dEpi, dorsal epidermis; dr, dorsal ridge; es, oesophagus; mal, Malpighian tubes; me, mesectoderm; ms, mesoderm; ol, optic lobes; pc, pole cells; ph, pharynx; pl, procephalic lobe; pm, posterior midgut; pNR, procephalic neurogenic region; pr, proctodeum; sg, salivary gland; tr, tracheae; vNR, ventral neurogenic region; C1–C3, gnathal segments; T1–T3, thoracic segments; A1–A10, abdominal segments.
their fate before the segregation of neuroblasts occurs? To answer this question, single ectodermal cells were transplanted from globally labelled wild-type donors into unlabelled wild-type hosts within an area corresponding to the anlagen of segments T3 and A1 (Technau & Campos-Ortega, 1986a). In the first experimental series, cells were transplanted homotypically between different dorsoventral levels of this area. The results correspond to the fate map. Transplanted dorsal ectodermal cells (1, see Fig. 1B) gave rise to progeny cells in the dorsal epidermis; in some cases a single pericardial cell was labelled. Such cells probably derived from the amnioserosa anlage which constitutes the dorsalmost area of the fate map. Cells from the dorsolateral ectoderm (2, see Fig. 1B) produced dorsolateral epidermis or sensory organs, which were labelled together with surrounding epidermal cells. Homotypically transplanted cells of the vNR (3, 4, 5, see Fig. 1B) gave rise to clones located either in the ventral epidermis (Fig. 2D,E) or in the CNS (Fig. 2A,B) or in both. The frequency of neural clones as compared to epidermal clones decreases from ventral to lateral levels, again reflecting the dorsoventral gradient of neurogenic abilities in the vNR. The occurrence of mixed epidermal/neural clones indicates the existence of common precursor cells for both lineage pathways. The SIII neuroblasts and most or all sensory organs are presumably derived from such precursors because they have already undergone one postblastoderm mitosis before their segregation.

Epidermoblasts and neuroblasts not only produce completely different histotypes but they also differ with respect to their proliferative capabilities. Epidermoblasts produce up to eight daughter cells which corresponds to three symmetrical mitoses. This is in agreement with histological studies conducted by Hartenstein & Campos-Ortega (1985). Sizes of neural clones, on the other hand, vary between 2 and 28 cells. Neuroblasts are stem cells, which bud off a series of smaller ganglion mother cells by asymmetrical divisions. In the grasshopper, each ganglion mother cell then divides once to produce two cells, which later differentiate into neurones (Goodman & Spitzer, 1979). Assuming the same behaviour for ganglion mother cells in Drosophila, neuroblasts would be capable of performing up to 14 mitoses. On the other hand, the analysis of the global mitotic pattern and cell counts on whole mounts of embryos at successive stages of postblastoderm proliferation, predict five to eight divisions for each neuroblast (Hartenstein, Rudloff & Campos-Ortega, 1987). This discrepancy may arise from several still unknown circumstances, e.g. the effects of the transplantation procedure on the proliferative abilities of the neuroblasts, the role of naturally occurring cell death and the proliferative behaviour of ganglion mother cells. However, it could also be that the transplanted cell divides while still in the ectoderm giving rise to two neuroblasts.

In a second experimental series, individual cells were transplanted heterotopically from ventral to dorsal ectodermal positions and vice versa. Such transplantations within the anlagen of T3 and A1 uncovered a difference in the behaviour of ventral and dorsal cells. When cells from the vNR were transplanted into the anlage of the dorsal epidermis (5→1, see Fig. 1B), they developed according to their origin. They gave rise either to epidermal or neural clones, the latter showing all characteristics of CNS clones (subepidermal clusters of small, spherical cells with clearly differentiated axonal processes; Fig. 2C). Homotypically transplanted dorsal ectodermal cells, however, never gave rise to neural progeny. Neural clones also develop in the lumen of the gut, if their progenitor cells (derived from the vNR) were transplanted into the midgut anlage (4→10, see Fig. 1B). These ectopically growing neural progeny cells strongly suggest that their respective progenitor cells had already become committed for neurogenesis at the onset of gastrulation when the transplantations were performed. On the other hand, not all cells of the neurogenic ectoderm become committed that early: following homotopic transplantations of vNR-cells, the frequency of neural clones is much higher than just 25 %, the proportion of vNR cells actually segregating as neuroblasts during normal development. It appears that all cells of the neurogenic ectoderm have the capability to develop into neuroblasts, but that only some of them become committed to this fate at the onset of gastrulation. During normal development, the majority of the cells seems to be prevented from following this primary fate, thus being forced into the epidermal pathway. A primary neural fate of the cells within the neurogenic regions is also in agreement with the phenotype of several neurogenic mutants in which the cells of the neurogenic regions exclusively express the neural fate at the expense of the epidermal fate (Campos-Ortega, 1985).

Cells transplanted from the dorsal epidermal anlage into the vNR (1→5, see Fig. 1B), on the other hand, gave rise to progeny cells according to the position into which the cell was transplanted. In a ventral ectodermal position, dorsal cells may be induced to adopt also the neural fate, which demonstrates the hidden potency of these cells to form neural structures. Their pluripotency, however, seems to be restricted to ectodermal fates, since ectodermal cells when transplanted heterotopically into the endoderm (i.e. the midgut anlage) or mesoderm did not participate in the formation of the
endodermal or mesodermal derivatives. Most of these cells degenerated, although some of them developed autonomously in the lumen of the midgut or between other tissues. Cells from the neurogenic ectoderm developed into clusters of cells with long fibre projections and cells from the proctodeum anlage gave rise to clones of up to eight cells which were arranged in small rings (Fig. 2G). As tested at a later stage, cells from the dorsal epidermal anlage remain pluripotent at least until after their first postblastoderm mitosis.

The main conclusions to be drawn from these transplantation experiments with ectodermal cells of the wild type are (1) commitment of a particular ectodermal cell depends on interactions between that cell and its neighbours and (2) commitment of dorsal truncal ectodermal cells towards epidermogenesis seems to be delayed with respect to the commitment of vNR cells towards neurogenesis.

The essential role of cell communication in the process of the epidermal/neural dichotomy of ectodermal cells is emphasized by a series of transplantsations carried out with single cells from several neurogenic mutants into wild-type hosts (Technau & Campos-Ortega, 1987). In these neurogenic mutants, the mechanism leading to this dichotomy of cell fates is affected (Campos-Ortega, 1985). Since the purpose of this review is to focus on the clonal analysis of wild-type cells, studies on mutants will not be further discussed here.

(3) Clonal analysis of mesodermal derivatives

The cells of the mesoderm invaginate along the ventral midline (Fig. 1) to form the major part of the ventral furrow. The mesoderm gives rise to a variety of derivatives exhibiting very different histotypes. Regional differentiation of the mesodermal layer, i.e., formation of splanchno- and somatopleura and of the somatic muscles surrounding part of the posterior midgut (lateral view), laterally over three consecutive segments. (I) Visceral muscles (Fig. 21), fat body, macrophages and two additional cell types with cytoplasmic fibre projections and cells from the proctodeum anlage developed autonomously into ring-like or spherical clusters. (H) Somatic muscle cells comprising descendants of a homotopically transplanted cell of the vNR (4, see Fig. 1B). Their histotype as well as their subepidermal location strongly suggests that the transplanted progenitor cell was already committed for the neural pathway. (D,E) Epidermal clones comprising eight (maximum clone size) and four cell, respectively, each having developed from a homotypically transplanted cell of the vNR (4, see Fig. 1B). Cells become arranged along the dorsoventral axis but remain in close neighbourhood. (F,G) Progeny cells stemming from cells of the proctodeum anlage.

The lineages of single mesodermal cells were studied (Beer et al. 1987) following their homotypic transplantation (at about 50% EL, corresponding to anlagen T3/A1; 6, see Fig. 1B) from globally labelled donors into unlabelled hosts at the early gastrula stage. The features of the clones were analysed in the fully differentiated embryo. Labelled derivatives found in these embryos were somatic muscles (Fig. 2H), visceral muscles (Fig. 21), fat body, macrophages and two additional cell types with cytoplasmic processes (monopolar and multipolar cells). There were many cases of mixed somatic muscle/fat body clones, and relatively few mixed somatic muscle/visceral muscle clones or mixed clones comprising all three histotypes, which indicates the existence of common precursors for these histotypes. The spatial distribution of the member cells of a clone furthermore indicates that neither segmental nor midline restrictions exist for mesodermal cells: myoblasts of the same lineage may be found in somatic muscles that are distributed uni- or bilaterally across up to three segments (Fig. 2H).

Fig. 2. Examples of various ectodermal (A–G) and mesodermal (H,I) cell clones in whole mounts of embryos at a late stage. Clones originate from progenitors which were taken from donors that were globally labelled with HRP and were singly (except in G) transplanted into unlabelled hosts. In all cases, transplantation was performed approx. 10 min after the start of gastrulation, when donors and hosts had just completed celluarization (compare fate map, Fig. 1B).

Fixation and staining the progeny cells for HRP occurred after head involution (approx. 20 h after transplantation, at 18°C) of the host embryos. (A,B) Neural clones within the ventral nervous system following homotopic transplantation of a cell from the ventral neurogenic region (4, see Fig. 1B). The fibres in (A) project along one of the connectives (lateral view). (B) is a composite photograph (ventral view) showing a medially located clone with a motoneurone carrying bilateral projections which leave the CNS through segmental nerves (arrows). (C) Clone of two cells derived from a progenitor cell which was heterotopically transplanted from the vNR into the dorsal epidermal anlage (5—>1, see Fig. 1B). Their histotype as well as their subepidermal location strongly suggests that the transplanted progenitor cell was already committed for the neural pathway. (D—G) Epidermal clones comprising eight (maximum clone size) and four cells, respectively, each having developed from a homotypically transplanted cell of the vNR (4, see Fig. 1B). Cells become arranged along the dorsoventral axis but remain in close neighbourhood. (F,G) Progeny cells stemming from cells of the proctodeum anlage.

Whereas cells in (F) (lateral view) stem from a single progenitor which was homotypically transplanted from/to the proctodeum anlage (12, see Fig. 1B), those in (G) (composite photograph) originate from at least three hindgut progenitors which were heterotopically transplanted between cells of the mesoderm (12—>6, see Fig. 1B). In the first case the daughter cells became part of the hindgut wall epithelium. In the latter case cells did not develop according to their new position but developed autonomously into ring-like or spherical clusters. (H) Somatic muscle cells comprising descendants of a homotypically transplanted mesodermal cell from the ventral furrow (6, see Fig. 1B); the clone extends laterally over three consecutive segments. (I) Visceral muscles surrounding part of the posterior midgut (lateral view); the clone derives from a mesodermal precursor cell which was heterotopically transplanted between cells of the dorsal epidermal anlage (6—>2, see Fig. 1B). hg, hindgut; mg, midgut; vns, ventral nervous system; spg, supraoesophageal ganglion. Nomarski optics. Bar, 30 μm.
The progeny of mesodermal cells participated in the formation of up to nine somatic muscle cells. Clone sizes in the fat body varied from 1 to 16 cells and in the visceral musculature from 3 to 26 cells. Because of the syncytial nature of somatic muscles and since it is not known whether visceral muscles and fat body in *Drosophila* form syncytial structures, it is not possible to draw firm conclusions about the proliferative behaviour of the respective precursors from these results. Staining of the hosts before germ band shortening, i.e. before myoblasts fuse to form syncytia, showed the number of cells within mesodermal clones to vary between 1 and 7. This result is compatible with a maximum of three mitoses of mesodermal cells and agrees with the histological observations by Hartenstein & Campos-Ortega (1985), although it does not exclude the possibility of additional mitoses in mesodermal structures after germ band shortening.

The degree of commitment of mesodermal cells was investigated by heterotopic transplantations. Following transplantation of mesodermal cells from the ventral furrow into the anlagen of the anterior (6→9, see Fig. 1B) or posterior midgut (6→10, see Fig. 1B) or to the amnioserosa, the progeny cells did not participate in the formation of midgut or of amnioblasts; instead, they gave rise to mesodermal derivatives or they died. After transplantation into ectodermal regions, some of the cells gave rise to ectodermal derivatives. These cases are probably due to having transplanted ventral ectodermal rather than mesodermal cells, since these cells are in close contact and removal of one cell type from the ventral furrow is difficult to control. We therefore believe that cells from the mesoderm are committed to a mesodermal fate as early as the onset of gastrulation. Following heterotopic transplantation of mesodermal cells into the dorsolateral epidermal anlage (6→2, see Fig. 1B) these cells developed into visceral muscles much more frequently than when transplanted into the ventral neurogenic ectoderm or, homotopically, into the ventral furrow. This suggests that cells located dorsally in the mesodermal primordium preferentially adopt the visceral muscle fate. Cells heterotopically transplanted along the anterior–posterior axis of the ventral furrow (7→8, see Fig. 1B) gave rise to mesodermal clones of a size and extent comparable to those after homotopic transplantations. The types of labelled muscles were in accordance with the segments into which the progenies of the transplanted cell came to be located. This indicates that cells of the mesoderm are not committed to develop within the limits of a given segment, nor are they committed to give rise to any specific type of mesodermal structure.

(4) Clonal analysis of endodermal derivatives

The midgut develops from two separate anlagen, the anterior midgut anlage at the anterior egg pole and the posterior midgut anlage beneath the pole cells at the posterior pole (Fig. 1). The anterior midgut anlage was found to be subdivided into two areas which perform different morphogenetic movements (Technau & Campos-Ortega, 1985). The posterior ventral subdivision invaginates together with the mesoderm at gastrulation and forms the anterior tip of the ventral furrow. The anterior subdivision of the anterior midgut anlage invaginates much later, together with the stomodeum. The posterior midgut anlage invaginates dorsally at gastrulation to form part of the amnioproctodeal invagination.

Cells from both anlagen were transplanted homotopically from labelled donors into unlabelled hosts at the early gastrula stage (9 and 10, see Fig. 1B), when the posterior anlage has just separated from the posterior pole in an anterior and dorsal direction. Lineages of singly transplanted cells were analysed in the fully developed host embryo (Technau & Campos-Ortega, 1986b). Cells from both anlagen behaved essentially the same with respect to their proliferative abilities and their differentiation. Clone sizes generally varied between four and eight cells, which would correspond to two or three mitoses of a symmetrical type. This is in good agreement with the results of histological studies (Hartenstein & Campos-Ortega, 1985). The progeny cells were of two different histotypes. Cells of the first type formed part of the midgut wall epithelium (Fig. 3A,B). The other type was small spindle-like cells which were loosely associated with the inner surface of the midgut epithelium and which carry one to three short cytoplasmic processes. The clones either exclusively consisted of epithelial cells or both histotypes were represented, again suggesting a common ancestry of the two cell types.
After heterotopic transplantations of cells from the anterior to the posterior midgut anlage (9→10, see Fig. 1B), and vice versa, they produced progeny cells that participated in the formation of the respective midgut region. The morphological and lineage characteristics of such cells did not differ from those of homotopically transplanted cells. On the other hand, heterotopically transplanted midgut anlagen cells into the mesoderm or into the ectoderm were not able to differentiate into mesodermal or ectodermal derivatives. These experiments are consistent with a commitment of endodermal cells to produce midgut structures as early as the beginning of gastrulation. However, although they are located at opposite poles of the egg, cells of the two midgut anlagen are interchangeable; they do not seem to be committed to form exclusively anterior or posterior elements of the midgut.

(5) Clonal analysis of the pole cells

At the end of the 8th nuclear division, shortly before formation of the syncytial blastoderm, two to four cytoplasmic buds protrude from the posterior egg pole. Concurrently with nuclear divisions 9 and 10, these pole buds will undergo two consecutive divisions (Foe & Alberts, 1983). In order to achieve incorporation of the lineage marker into these cells, the marker has to be injected into donor embryos before pole bud formation (Technau, 1986). Movements, fate, proliferative abilities and commitment of the pole cells were studied by transplanting these cells into unlabelled hosts (Technau & Campos-Ortega, 1986b). Up to the gastrulation stage most of the initial pole cells perform another one or two mitoses, whereas at the same time the nuclei of prospective somatic cells perform three divisions (11th—13th). The final number of pole cells at gastrulation was found to vary between 23 and 52 (Zalokar & Erk, 1976; Underwood, Caulton, Illmensee & Mahowald, 1976; Mahowald, Illmensee & Turner, 1976; Underwood, Caulston, Allis & Mahowald, 1980). Homotopic transplantations of labelled pole cells (11, see Fig. 1B) clearly indicate that the pole cells exclusively participate in the formation of the gonads (Technau & Campos-Ortega, 1986b; Fig. 3C,D), which is in agreement with the conclusion of Underwood et al. (1980) using a similar experiment. Heterotopic transplantations of pole cells into endodermal (11→9), mesodermal (11→6), or ectodermal environments (11→4, see Fig. 1B) lend further support to a commitment of pole cells exclusively as germ line progenitors as early as they pinch off from the pole buds. The number of pole cells that become incorporated into each of the gonads varies between 7 and 13 and the differences in the number of pole cells between the left and the right gonad varies between 0 and 2. This suggests the existence of a mechanism that regulates the final number of pole cells in the gonads. In fact, in hosts that are supplied with an additional set of 15–30 pole cells the number of cells that become incorporated into the gonads remains in the same range as in the controls. Supernumerary pole cells that do not become incorporated get lost in the gut lumen, degenerate or may be excreted at later stages.

(6) Selected aspects of the development of progenitor cells from the different germ layers in comparison

(6.1) The topology of precursors within the germ layers

Cells of the mesoderm and of the ectoderm give rise to a variety of different histotypes. The progenitor cells within these germ layers are not arranged according to a unique pattern. Within the ectoderm the neurogenic regions, the dorsal epidermis and the fore- and hindgut, represent separate anlagen. Within the neurogenic regions, progenitor cells of two morphologically and functionally extremely different structures are intermingled according to a ‘salt and pepper’ strategy, presumptive epidermoblasts being in the immediate neighbourhood of presumptive neuroblasts. This pattern requires a separation of the two types of cells by segregation of single cells from a contiguous sheath. The mechanisms of commitment of cells within such an anlage must be different, i.e. more complex, than within anlagen in which all cells participate in the formation of the same structure (e.g. foregut). The topology of the different progenitors within the mesoderm also seems to represent a complex pattern, but this is only poorly understood. With respect to the distribution of muscle progenitors it seems that the ventralmost area of the cellular blastoderm, which will form the roof of the ventral furrow, is composed of a mixture of cells...
which will contribute to the formation of either the visceral or dorsal somatic musculature or both, whereas the area of the mesoderm that invaginates last and which remains in contact with the ectoderm, almost exclusively gives rise to ventral somatic musculature. Precursors of the other mesodermal derivatives seem to be distributed over the entire mesoderm in an unknown fashion.

(6.2) Common precursors for different histotypes

In all three germ layers some progenitor cells were found to give rise to mixed clones comprising cells of different histotypes (Fig. 4). Several progenitors within the ectoderm give rise to epidermal cells as well as to neural cells in the CNS or in sensory organs. Mesodermal cells produce clones that participate in the formation of somatic and visceral musculature, as well as in the formation of fat body or other mesodermal derivatives. Endodermal cells in many cases gave rise to both epithelial and spindle-like cells. In all of these cases the decision of cells to follow a given pathway seems to occur rather late, i.e. following the first or second postblastoderm mitosis. The germ line precursors (pole cells) and the cells of the amnioserosa anlage, on the other hand, develop only one histotype each.

(6.3) Proliferative performances

Most of the larval progenitor cells perform up to three mitoses, as judged from the maximum clone sizes (Fig. 4) obtained following their transplantation at the beginning of gastrulation (Technau & Campos-Ortega, 1986a,b; Beer et al. 1987). This agrees well with histological studies showing that three postblastoderm mitotic waves pass through almost all larval primordia (Hartenstein & Campos-Ortega, 1985). The only cells found that do not perform any postblastoderm mitosis during embryogenesis are the

Fig. 4. Lineage relationships and commitment of developmental pathways in the Drosophila embryo. Numbers marked with * represent maximum clone sizes obtained from singly transplanted cells of the respective anlagen.
pole cells (at least up to the formation of the gonads) and the cells of the amnioserosa anlage. The precursors of the larval CNS, on the other hand, may divide more than three times. Normal histological observations indicate that all neuroblasts stereotypically perform five to eight mitoses, depending on the time of segregation from the ectoderm (SI–III subpopulations of neuroblasts; Hartenstein & Campos-Ortega, 1984; Hartenstein et al. 1987). Single transplanted neural precursors, on the other hand, produced clone sizes that varied between 2 and 28 cells (Technau & Campos-Ortega, 1986a, 1987), corresponding to 1–14 divisions of a stem cell mode. An explanation for this discrepancy may be that some of the transplanted cells perform their first division while still being in the ectoderm and that both daughter cells then segregate as neuroblasts. A further discrepancy between the available data concerns the mitotic activity of the hindgut progenitors. Whereas histological studies, as well as lineage studies on singly transplanted cells, suggest up to three postblastoderm mitoses for hindgut cells, mosaic studies from Janning, Lutz & Wissen (1986) suggest up to five mitoses for the cells forming the Malpighian tubules. Most of the mesodermal cells divide three times, as deduced from histological observations. Clone sizes within the somatic musculature and within mesodermal clones before the cells fuse to form syncytia are also consistent with up to three postblastoderm mitoses for the mesoderm. Since it is not known whether visceral myoblasts or fat body cells form syncytial structures, clone sizes obtained in these organs are difficult to interpret with respect to the proliferative behaviour of the transplanted progenitors.

(6.4) The degree of commitment

The heterotopic transplantations of single labelled progenitor cells between the different anlagen indicate that ectodermal, mesodermal and endodermal lineages are separated at least from the beginning of gastrulation (Fig. 4). Pole cells are already committed as germ line precursors from the moment of their appearance at the posterior pole at the early syncitial blastoderm stage. Heterotopic transplantations of cells from/to various positions within the germ layers demonstrate that, immediately after cell formation, cells are not yet committed for the different histotypes of a given germ layer, with the possible exception of some cells within the neurogenic ectoderm, which seem to be committed as presumptive neuroblasts already at early gastrula stages.

(6.5) Morphogenetic movements and the distribution of clonally related cells with respect to segment boundaries

The spatial distribution of the member cells of a given clone between different structures reflect the morphogenetic movements performed by these cells relative to each other and relative to the segment boundaries. The extent of these movements drastically differs between the germ layers, being minimal for ectodermal clones and largest for endodermal clones. Generally, the cells of an ectodermal clone remain in close contact. CNS clones are dense clusters of spherical cells (Fig. 2A–C); occasionally, glia cells may become separated by a few cell diameters from neurones within the same clone and occupy positions near the neurilemma. Cells of a truncal epidermal clone become arranged in dorsoventrally oriented rows (Fig. 2D,E), presumably due to the dorsal movement of the epidermal primordium during dorsal closure. All ectodermal clones found, except those of the gut, were within the range of a particular segment or parasegment. This is also true for mixed epidermal/neural clones (unpublished results). Whether they are even restricted to a particular compartment has yet to be tested. Daughter cells within mesodermal and endodermal clones, on the other hand, almost always become intermingled with cells of other clones and do not respect segment boundaries. Clonally related mesodermal cells in somatic muscles uni- or bilaterally were found to be distributed over up to three segments (Fig. 2H). Cells of clones in the endodermal midgut were distributed over either the anterior or the posterior half of the midgut (Fig. 3A), depending on whether their progenitors were transplanted into the anterior or into the posterior midgut anlage.

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